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# Growth Temperature Effects on Thylakoid Membrane Lipid and Protein Content of Pea Chloroplasts[1]

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## ABSTRACT

**The lipid composition and level of unsaturation of fatty acids has been determined for chloroplast thylakoid membranes isolated from *Pisum sativum* grown under cold (4°/7°C) or warm (14°/17°C) conditions. Both the relative amounts of lipid classes and degree of saturation were not greatly changed for the two growth conditions. In cold-grown plants, there was a slightly higher linolenic and lower linoleic acid content for the glycolipids monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), and sulfoquinovosyldiacylglycerol. In contrast to thylakoid membranes, a non-thylakoid leaf membrane fraction including the chloroplast envelope, had a higher overall level of fatty acid unsaturation in cold-grown plants due mainly to an increase in the linolenic acid content of MGDG, DGDG, phosphatidylglycerol, and phosphatidylcholine. The most clear cut change in the thylakoid membrane composition was the lipid to protein ratio which was higher in the cold-grown plants.**

A remarkable feature of the thylakoid membrane of higher plants is the very high degree of unsaturation of the lipid acyl chains (18). It is this feature which must largely account for the relatively high fluidity of the thylakoid membrane when compared with other membranes (12, 13). The reason for this special property is becoming more evident as our understanding of photosynthetic electron transport advances. Recent work suggests that the majority of PSI and PSII are laterally separated into different regions of the thylakoid, with the PSII predominantly in the appressed membranes of the grana and PSI in the nonappressed membranes having external surfaces exposed to the stroma (1, 2, 6). This model focuses attention on plastoquinone as a long range diffusing redox agent dependent for its function on the physical state of the lipid matrix (25). At the level of energy transfer, it also seems that the regulation of State 1-State 2 transitions (4) is governed by lateral diffusion, except in this case it is the slower movement of large pigment-protein complexes which are involved (5).

As the temperature is lowered, the fluidity of the membrane will drop and diffusional processes will be hindered. Many plants can, however, photosynthesize and grow at low temperatures and this adaptation could partly be due to changes in thylakoid membrane composition which optimizes the fluidity. Certainly, some analyses of leaf total lipids suggest that plants which are subjected to cold growth conditions do regulate the degree of unsaturation of their fatty acids, at least to a small extent (8, 19, 22). In this paper, we have concentrated our attention on the lipid and protein content of thylakoid membranes isolated from the cold resistant plant *Pisum sativum* grown at cold and warm temperatures.

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## MATERIALS AND METHODS

**Plant Material.** Pea seedlings (*Pisum sativum* L. var Feltham First) were grown from seed in trays of vermiculite in controlled environment cabinets (Fisons model 600G3/TTL) with a light intensity of 60 w/m$^2$, a 16-h photoperiod, and temperature regimes of either 4°/7°C or 14°/17°C average daily minimum/maximum. The daily watering cycle included the use of 'Long Ashton' nutrient solution (15) every 7th d. Second and third leaf pairs were harvested from seedlings which had reached a developmental stage characterized by expansion of the third leaf pair.

**Isolation of Membrane Fractions.** Membrane fractions were prepared using the same procedure as described previously (8), except that after homogenization of leaves the brei was filtered through 10 layers of muslin and two of 25-μm mesh nylon, and the differential centrifugation of thylakoid and non-thylakoid fractions involved just two spin speeds: 5000*g* and 80,000*g*.

**Extraction and Analysis of Protein and Lipid.** Lipids were extracted by the method of Williams and Merrilees (24), protein was determined by the Lowry method (20) after extraction of pigments in 80% acetone containing 50 mM NaCl, and Chl concentrations were determined according to Arnon (3). Lipid classes were separated by TLC and fatty acid methyl esters analyzed as described previously (8). Quantities of lipid classes and fatty acids in membrane fractions were corrected for the presence of thylakoids in the 80,000*g* fraction with the assumption that Chl in this fraction was associated with a quantity of thylakoid fatty acid which could be estimated from the fatty acid to Chl mole ratio determined for the 5,000*g* fraction. For this correction, it was assumed that the Chl in the 80,000*g* fraction was associated with the same specific lipids as Chl in the thylakoid fraction. Lipid class and fatty acid compositions for 'thylakoid' and 'non-thylakoid' were calculated after this correction.

Results are given as average values obtained from different batches of plants with SE given where a sufficiently large number of batches were analyzed. For fatty acid composition of individual lipid classes and relative amounts of different lipid classes, average values from two batches of plants were calculated. Previous experience (*e.g.* 8) has shown that for fatty acid unsaturation, expressed as average number of double bonds per lipid molecule, SE are typically less than 2% of average values for glycolipids and 5% for phospholipids; and for relative amounts of different lipid classes, typically less than 5% of average values. The differences between duplicates in the results reported here were consistent with this degree of variation.

## RESULTS

Thylakoids isolated from pea plants grown at the lower temperatures had a higher lipid content relative to Chl and protein than the thylakoids of warm temperature plants (Table I). As Table II shows, fatty acid analysis of the isolated thylakoid

Table I. *Relative Amounts of Lipid, Protein, and Chl in Thylakoids of Plants Grown in Cold or Warm Conditions*

Weight to weight ratios are given as average values from analysis of separate batches of plants; numbers are given in parentheses.

| Growth Conditions | Lipid/Chl | Protein/Chl | Lipid/ Protein |
|---|---|---|---|
| Cold | 2.99 ± 0.05 (8) | 4.65 ± 0.40 (6) | 0.64 |
| Warm | 2.68 ± 0.06 (10) | 4.60 ± 0.46 (6) | 0.58 |

membranes indicated that there was no large difference in cold- and warm-grown plants in terms of the overall unsaturation level (as determined as the average number of double bonds per lipid molecule) or in terms of the relative amounts of each fatty acid type. A *t* test analysis of the fatty acid data in Table II does, however, suggest that there may be a slightly higher linolenic acid (+1.5%, $P < 0.05$) and lower linoleic acid (−1.0%, $P > 0.05$) content in colder-grown plants compared with those grown in the warmer conditions.

Thylakoid lipid extracts were separated into individual lipid classes by TLC and fatty acid compositions determined for each. None of the lipid classes had large differences in fatty acid composition after cold and warm growth (Table III), but a general trend for the cold-grown plants was that of slightly higher linolenic acid and lower linoleic acid percentages, particularly for the glycolipids.

After centrifugation to give thylakoid samples, the supernatant was removed and centrifuged at 80,000*g* to give a membrane preparation containing other cell membranes, including chloroplast envelopes and some contaminating thylakoids. The lipids of this predominantly non-thylakoid sample were analyzed and a correction made for the presence of contaminating thylakoid membranes on the basis of Chl (Table IV). The correction for the contamination was for about 10% of the total Chl extracted and the thylakoid to non-thylakoid lipid ratio was 4 to 1 for both cold- and warm-grown plants.

Comparison of Tables III and IV indicates that the non-thylakoid fractions have lower degrees of unsaturation than the thylakoid membranes as expressed by the average number of double bonds per lipid molecule. Moreover, in non-thylakoid preparations from cold-grown plants, there was a higher level of unsaturation than for warm-grown plants in the lipid classes MGDG[2], DGDG, PG, and PC.

The relative amounts of each lipid class are given in Table V for both thylakoid and non-thylakoid material. Growth conditions had no obvious effect on lipid class composition of thylakoids or on the relative amounts of glycolipids and PG of non-thylakoid membranes. However, it seemed that PC was a higher percentage of total lipid in the non-thylakoid preparation from cold-grown compared with warm-grown plants and there was a lower amount of the other phospholipid fraction (predominantly phosphatidylethanolamine).

[2] Abbreviations: MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine; SQDG, sulphoquinovosyldiacylglycerol.

Table II. *Fatty Acid Composition of Total Acyl Lipids Extracted from Thylakoid Membranes of Plants Grown in Cold or Warm Conditions*

Average values with SE are given for analysis of separate batches of plants; numbers of samples (*n*) are given in the table.

| Growth Conditions | | Fatty Acid Composition | | | | | | Avg. No. of Double Bonds/ Lipid Molecule |
|---|---|---|---|---|---|---|---|---|
| | | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | |
| | *n* | *mol %* | | | | | | |
| Cold | 8 | 10.4 ± 0.4 | 2.3 ± 0.1 | 1.6 ± 0.2 | 1.8 ± 0.2 | 4.1 ± 0.3 | 80.9 ± 0.5 | 5.106 ± 0.028 |
| Warm | 10 | 9.6 ± 0.4 | 2.1 ± 0.1 | 1.8 ± 0.1 | 2.1 ± 0.2 | 5.1 ± 0.3 | 79.4 ± 0.5 | 5.050 ± 0.024 |

Table III. *Comparison of Fatty Acid Compositions of Lipid Classes Extracted from Chloroplast Thylakoids of Plants Grown in Cold and Warm Conditions.*

Average values are given for two separate batches of plants.

| | Growth Temperature | Fatty Acid Composition | | | | | | Saturated Fatty Acids | Avg. No. of Double Bonds/ Lipid Molecule |
|---|---|---|---|---|---|---|---|---|---|
| | | 16:0[a] | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | | |
| | | *mol %* | | | | | | *%* | |
| MGDG | C | 2.1 | | 0.6 | 0.9 | 4.2 | 92.2 | 2.7 | 5.715 |
| | W | 2.3 | | 0.7 | 1.0 | 6.0 | 90.0 | 3.0 | 5.661 |
| DGDG | C | 8.1 | | 2.4 | 1.1 | 2.1 | 86.4 | 10.5 | 5.285 |
| | W | 9.2 | | 2.9 | 1.4 | 4.2 | 82.3 | 12.1 | 5.138 |
| SQDG | C | 24.6 | | 4.6 | 2.4 | 6.1 | 62.3 | 29.2 | 4.034 |
| | W | 24.8 | | 4.8 | 2.9 | 8.7 | 58.8 | 29.6 | 3.941 |
| PG | C | 29.0 | 16.5 | 2.9 | 7.3 | 9.6 | 34.9 | 31.9 | 2.949 |
| | W | 29.9 | 14.6 | 2.7 | 8.9 | 9.9 | 33.9 | 32.6 | 2.900 |
| PC | C | 23.7 | | 6.3 | 4.3 | 33.0 | 32.8 | 30.0 | 3.372 |
| | W | 23.6 | | 6.6 | 5.3 | 32.9 | 31.7 | 30.2 | 3.321 |
| Other phospholipids | C | 30.0 | | 7.0 | 4.7 | 26.0 | 32.0 | 37.0 | 3.079 |
| | W | 20.8 | | 11.3 | 12.3 | 25.3 | 30.5 | 32.1 | 3.083 |

[a] Fatty acids denoted by number of carbon atoms:number of double bonds.

Table IV. *Comparison of Fatty Acid Compositions of Lipid Classes Extracted from the 80,000g Membrane Fraction Prepared as Described in "Materials and Methods" from Plants Grown in Cold and Warm Conditions*

Average values are given for two separate batches of plants.

| | Growth Temperature | Fatty Acid Composition | | | | | | Saturated Fatty Acids | Avg. No. of Double Bonds/ Lipid Molecule |
|---|---|---|---|---|---|---|---|---|---|
| | | 16:0[a] | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | | |
| | | *mol %* | | | | | | % | |
| MGDG | C | 9.0 | | 2.4 | 3.9 | 8.3 | 76.4 | 11.4 | 4.995 |
| | W | 11.6 | | 3.0 | 3.3 | 10.6 | 71.6 | 14.5 | 4.766 |
| DGDG | C | 18.3 | | 5.7 | 1.0 | 7.7 | 67.4 | 24.0 | 4.369 |
| | W | 21.2 | | 7.8 | 1.7 | 10.6 | 58.8 | 29.0 | 3.981 |
| SQDG | C | 42.9 | | 5.5 | 2.1 | 20.0 | 29.5 | 48.4 | 2.612 |
| | W | 34.7 | | 6.9 | 2.9 | 19.9 | 35.6 | 41.6 | 2.990 |
| PG | C | 36.9 | 13.5 | 2.6 | 3.4 | 17.3 | 26.4 | 39.5 | 2.613 |
| | W | 37.2 | 11.6 | 3.6 | 8.0 | 16.8 | 22.8 | 40.8 | 2.430 |
| PC | C | 24.6 | | 5.1 | 2.5 | 36.8 | 31.2 | 29.7 | 3.388 |
| | W | 27.4 | | 6.6 | 3.1 | 38.4 | 24.6 | 34.0 | 3.074 |
| Other phospholipids | C | 40.5 | | 5.3 | 1.6 | 35.2 | 17.5 | 45.8 | 2.486 |
| | W | 33.9 | | 5.3 | 1.7 | 40.6 | 18.7 | 39.2 | 2.772 |

[a] Fatty acids denoted by number of carbon atoms:number of double bonds.

Table V. *Relative Amounts of Each Lipid Class in Thylakoid and Non-Thylakoid Membrane Preparations from Cold- and Warm-Grown Plants*

Values are averages from analysis of two separate batches of plants.

| Lipid Class | Thylakoid | | Non-Thylakoid | |
|---|---|---|---|---|
| | Cold | Warm | Cold | Warm |
| | *mol %* | | | |
| MGDG | 40 | 42 | 9 | 10 |
| DGDG | 26 | 26 | 16 | 17 |
| SQDG | 10 | 9 | 7 | 6 |
| PG | 11 | 11 | 8 | 8 |
| PC | 8 | 7 | 44 | 38 |
| Other phospholipids | 5 | 6 | 16 | 21 |

## DISCUSSION

There are many reports from studies of a wide variety of membrane types which show that temperature adaptation is linked to changes in unsaturation of fatty acids (e.g. 9–11). A greater degree of acyl chain unsaturation is expected to result in a more fluid membrane (7) although this may not always apply because the degree of lipid ordering will also be affected by the presence of integral proteins and of sterols (17). In our investigations of the pea thylakoid membrane, we have not found that, for growth temperatures used, there is a change in the overall fatty acid composition of the magnitude reported for other membrane systems (9–11). Even for the individual lipid classes, only small differences were found in fatty acid levels for the cold and warm growth conditions. These results are consistent with our previous study when lipid analyses were conducted on pea plants grown in summer and winter conditions (8). In both studies, we noted small changes which may or may not be functionally significant. It is worth, however, noting Lyons' and Asmundson's (21) contention that, in the special case of fatty acid mixtures as highly unsaturated as those found in the thylakoid membrane, there can be significant effects on the physical properties of the mixture for only a small change in the degree of unsaturation.

The very small effect of growth at chilling temperatures on the fatty acids of thylakoid lipids contrasts with larger changes during acclimation to heat stress conditions (23), and appears to be in conflict with previous reports of distinct effects in total lipids extracted from leaves (8, 19, 22). The thylakoid acyl lipids are a major component of the total leaf membrane lipid and thus might be expected to show the same trends. However, the anomaly can be explained partly because the non-thylakoid membrane lipid fraction does vary its unsaturation level in response to temperature, and because a total leaf fatty acid analysis does not distinguish between membrane and non-membrane lipids. Recent studies by Nordby and Yelenosky (22) with citrus leaves have shown a large low temperature-induced increase in the unsaturation level of triglycerides but relatively small changes in polar lipids.

The non-thylakoid fraction that we have analyzed contains significant quantities of galactolipids suggesting that the chloroplast envelope is a component of this extract. The difference in the unsaturation levels of the non-thylakoid galactolipids (Table IV) indicate that the envelope lipids are more affected by growth temperatures than the thylakoid lipids, a conclusion already given for pea plants grown in winter and summer conditions (8).

The properties of the lipid matrix of membranes can be altered not only by changes in fatty acid compositions but also by changes in the relative amounts of individual lipid classes (7). We have found, however, that the response to growth temperature does not involve a change in relative amounts of lipid classes in thylakoids although a difference was observed in the non-thylakoid fraction in terms of amounts of PC and minor phospholipids. The non-thylakoid membrane fraction, of course, contains more than one type of membrane and, therefore, it is possible that the lipid class variation is due to changes in relative amounts of different membranes.

Our study has shown that, although there is only a minor change in the lipid composition of pea thylakoid membranes in response to changes in growth temperature, there seems to be a change in the lipid to protein ratio (Table I). This latter observation may be important since the lipid to protein ratio could be one of the factors which contributes to regulation of fluidity in this particular membrane. An increase in lipid to protein ratio almost certainly leads to a more fluid membrane as shown both for intact

(16) and fragmented (14) thylakoids. A strategy of maintaining a stable high fatty acid unsaturation and regulating membrane viscosity by altering the relative levels of proteins and lipids may have distinct advantages for the photosynthetic membrane. In this way, it will maintain a high fluidity and at the same time optimize the available amount of functional protein per unit area for a particular growth condition.

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